Welsh Histocompatibility & Immunogenetics Service (WHAIS)

USER GUIDE

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WTAIL.ORG.UK

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Contact Details

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Consultant Clinical Scientists

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Introduction

The Welsh Histocompatibility and Immunogenetics Service (WHAIS), together with the Welsh Bone Marrow Donor Registry (WBMDR), form the Welsh Transplantation and Immunogenetics Laboratory (WTAIL) and hosts the UK National External Quality Assessment Services for Histocompatibility and Immunogenetics (UK NEQAS for H&I).

WHAIS provides Histocompatibility and Immunogenetics (H & I) support to a variety of health care providers ranging from specialist transplant units to General Practitioners. This service is critical to both solid organ and blood stem cell transplant programmes. The services provided include: HLA typing, patient/donor crossmatching, HLA antibody testing, solid organ and stem cell donor/recipient matching, platelet typing and platelet antibody testing. Our staff work closely with clinicians in the management of solid organ transplant, blood stem cell transplant and thrombocytopenic patients.

Service Provision

Histocompatibility Testing for Solid Organ Transplantation

- WHAIS provides laboratory testing and clinical advice for patients requiring a pancreas or kidney who are transplanted in Wales The service supports transplant of various donor types including: Local deceased donors
- Deceased donors allocated via NHSBT Organ Donation and Transplant (ODT)
- Potential living renal donors
- ABO incompatible transplants
- HLA incompatible transplants
- Altruistic donor transplants
- Paired-pool exchange donors

Laboratory testing encompasses HLA typing, crossmatching and HLA antibody detection/identification. HLA typing is performed by PCR- based methods at the resolution required for patient registration and donor allocation by NHSBT ODT. Additional typing is performed to assist in antibody definition.

HLA antibody screening is routinely performed on potential transplant patients every 3 months, and following any sensitisation events, by CDC and Luminex based methods. Testing schedules for patients receiving antibody incompatible transplants are discussed and agreed with the transplant team on an individual basis. Donor specific antibody testing is performed for transplant patients where antibody mediated rejection is suspected and to monitor effectiveness of treatment.

Allogeneic and autologous crossmatching is performed by Complement Dependent Cytotoxicity (CDC) and Flow Cytometry.

The Laboratory manages patient registration and updates with ODT.
Clinical advice in relation to risk and donor selection is provided.

Histocompatibilty Testing for Haematopoietic Stem Cell Transplants

WHAIS supports services to the local transplant centre for autologous and allogeneic stem cell transplants from related and unrelated donors HLA-typing of patients and potential donors is performed by PCR-based methods and serology. If required the patient is tested for HLA antibodies by Luminex based methods.
A graft identification advisory service is provided and WHAIS liaise with WBMDR staff to facilitate unrelated donor, searches, confirmatory typing sample provision and stem cell products. WBMDR is licensed by the Human Tissue Authority to import haemopoietic stem cells.

Platelet Immunology Service

The laboratory provides investigations for a variety of immune thrombocytopenias which include:

- Platelet refractoriness
- Neonatal alloimmune thrombocytopenia
- Drug induced thrombocytopenia (excluding Heparin)
- Idiopathic thrombocytopenia (ITP)
- Gestational ITP
- Post transfusion purpura

HLA and HPA antibody screening is performed by Luminex based methods and the Monoclonal Antibody Immobilization of Platelet Antigen (MAIPA) test, respectively. HLA and HPA typing is performed by PCR-based methods.
The Laboratory works in close collaboration with the Welsh Blood Service, selecting suitable HLA/HPA matched platelets when clinically required.

Disease and drug hypersensitivity

HLA typing is provided to support disease diagnosis for a number of diseases e.g. HLA B27 and ankylosing spondylitis, DQA/DQB for coeliac disease and HLA*5701 for abacavir sensitivity.

Transfusion Related Acute Lung Injury
Investigations for this include HLA antibody screening of the implicated donors and the patient using Luminex technology and HLA typing where appropriate. Investigations for HNA antibodies are referred to the NHSBT.

Miscellaneous

The laboratory is happy to discuss requirement for ad-hoc HLA typing or antibody definition e.g. for appropriate research projects. Please contact Dr T Rees for further information.

Service Provision

Laboratory Tests

The current range that can be undertaken are given below

1. **Serological Phenotyping Tests**

- **HLA-A, -B, -C, -DR, -DQ phenotyping**
  Serological typing of HLA Class I (A, B & C) and II (DR DQ) specificities using the complement dependent cytotoxicity (CDC) assay for the purpose of assisting in the definition of expression variants.

2. **Serological Crossmatching and Antibody Screening Tests**

- **Complement dependent cytotoxic crossmatch**
  Complement dependent cytotoxicity crossmatch (CDCXM) to detect donor or auto reactive specific cytotoxic antibodies in recipient sera using donor lymphocytes. This technique uses purified B cells, T cells or peripheral blood lymphocytes to detect complement fixing antibody. This can also be performed using dithiothrietiol treated sera to determine the IgG or IgM class of the antibody. Donor specific HLA antibodies detected using this technique are normally considered a contraindication to solid organ transplantation.

- **Flow cytometry crossmatch (FCXM)**
  This is a more sensitive crossmatch than CDC and uses donor or recipient lymphocytes and recipient sera using a flurochrome labelled secondary antibody in order to detect IgG donor-specific antibodies against T and/or B cells. Donor specific antibodies detected using these techniques are considered an intermediate risk to transplant to transplant 'high risk' transplants.

- **Serum screening for HLA Class I and II antibodies**
  Complement dependent cytotoxicity (CDC) assay to determine the presence of specific HLA-A, -B and -Cw antibodies using an HLA-typed reference panel representing known specificities and incorporating uncommon antigen associations. HLA -DR and -DQ antibodies are specified using a random panel of HLA typed purified B cells. Donor specific antibodies detected using this technique are normally considered a contraindication to solid organ transplantation.

- **Serum screening for HLA Class I & Class II antibodies by Luminex based methods**
  This is specific for IgG HLA class I and/or II antibodies, including both complement and non-complement fixing sub classes, utilising Luminex

technologies. This technique is more sensitive that CDC, is highly specific and can resolve complex antibody profiles in highly sensitised patients using beads coated with a single HLA antigen. This technique is used to detect and specify antibodies in solid organ recipients, potential HLA mismatched and HSCT recipients, platelet refractoriness and for monitoring HLA antibodies in HLA antibody incompatible transplants.

- **Indirect PIFT-FC (Platelet Immunofluorescence Test by Flow Cytometry)**
  This technique uses a fluorescent anti-human antibody (AHG) to detect antibody bound to the platelet surface. Therefore all antibodies bound will be detected, i.e. HLA, ABO and HPA antibodies. It is often difficult to differentiate an HPA antibody using this technique because HLA antibodies can 'mask' the reaction pattern of HPA antibodies.

- **Direct PIFT-FC**
  Patient platelets are incubated directly with fluorescent AHG. The fluorescent level observed is compared to that of ten 'normal' platelet samples. The results indicate if there are raised levels of bound immunoglobulin on the patient's platelets.

- **MAIPA (Monoclonal Antibody Immobilisation of Platelet Antigen)**
  This sensitive technique detects antibodies directed to different platelet glycoproteins. This allows the identification of mixtures of platelet specific antibodies in the presence of HLA antibodies. The patient's serum is incubated with a panel of HPA typed platelets and defined antibodies reported.

3. **DNA-based HLA and HPA typing**

- **HLA Class I & II DNA typing: low resolution by PCR using sequence specific primers (PCR-SSP)**
  Determination of HLA-A, -B, -C,-DR, -DQ and DP alleles or groups of alleles by DNA analysis with a range of PCR primers giving definition comparable to serological typing, for the purpose of HLA typing in solid organ transplantation, as an aid to disease diagnosis and for selection of HLA compatible platelet transfusions.

- **HLA Class I & II DNA typing: high resolution (2nd field)**
  Determination of HLA-A, -B, -C,-DR, -DQ and -DP alleles by DNA analysis with a range of PCR primers or sequence based methods giving high resolution definition. This is used for matching in haemopoietic stem cell transplantation.

- **Human Platelet Antigen (HPA) Typing**
  Determination of HPA 'antigens' by typing for HPA alleles by DNA analysis using PCR-SSP.

- **HLA B*57:01 typing by molecular techniques**
  Determination of HLA-B57:01 (positive or negative) and differentiation of the 'common' HLA B57 subtypes (subtypes determined by sequence based typing). This is used to determine patients at risk of hypersensitivity type reactions to Abacavir.

4. Additional tests

- **HLA-B27 typing by flow cytometry**
  Determination of HLA-B27 (positive or negative) and differentiation of the common HLA-B27 subtypes 'B*27:02', 'B*27:05' and B*27:08 (subtypes 'B*27:02' and B*27:08 confirmed by PCR-SSP).
  Associations of HLA antigens with rheumatic diseases have been well established over the last two decades. Notably HLA-B27 typing has been particularly useful in the diagnosis of the atypical spondyloarthropathies.
  Determination of patient HLA-B27 status by flow cytometry (Coates & Darke, *European Journal of Immunogenetics* 1998,**25**,29).

- **Miscellaneous disease association**
  In consultation with our customers we can undertake any specific HLA typing request, or family studies. We also undertake disease susceptibility/association projects.
  Determination of HLA-A, B, C, DRB1/3/4/5, DQA1/DQB1, DPB1 HLA types in combinations and resolution appropriate to requirements.

- **ABO titres**
  This is performed using donor cells and recipient plasma for assessing and monitoring ABO antibody removal for ABO incompatible transplants. A diamed gel card method is used to determine titres by saline and AHG.

In addition to these tests, expert H&I advice is available from our staff.

Laboratory Hours

Routine

Monday to Friday 09.00-17.00 (excluding Bank Holidays)

Out of Hours Requests

The H&I service for deceased kidney and pancreas donor HLA typing, crossmatching and matching is available 24 hours a day, 365 days a year.

The out of hours service is staffed by Clinical Scientists, all of whom are employed within the WTAIL.

For each on call period two laboratory staff are available. This team consists of experienced Clinical Scientists who perform HLA typing and patient/donor crossmatching.

Contact Arrangements out of hours

On-call WTAIL Scientist

Contact WBS main switchboard (01443 622000) and ask for either the 'HLA Typer' or 'Crossmatcher' on-call for WTAIL.

Consultant advice may be obtained from the Head of WTAIL or the Consultant to the WTAIL.

Duty Consultant for WBS

Contact WBS main switchboard (01443 62200) and ask for the WBS Duty Consultant.

Sample Requirements and Reporting

Request Forms

Please use the standard WTAIL request forms as detailed below and complete ALL sections as appropriate (click on the links below to access the appropriate form):

HLA Typing Request Form - Renal
This form is to be used for all renal and pancreas patient/donor testing requests

HLA Typing Request Form –Haematopoietic Stem Cell Transplants (HSCT)
This form is to be used for all bone marrow patient/donor testing requests. For potential HSCT patients the diagnosis should be detailed on the request form. The relationship of family members as potential donors to the patient should be documented on the request form.

HLA & Disease Association Request Form
This form is to be used for all disease association requests, including requests for HLA-B27 and B*57:01 testing

Platelet Immunology and additional requests
Requests for platelet, TRALI and additional services can be made using standard haematology forms. The forms must clearly state the required investigation and referral laboratory in addition to the information required below.

Known high risk samples must be labelled as such on both the sample tubes and request form.

The following information is required on each sample tube:

Details (Please note Patient details on sample & request form must match exactly)	**Requirement**	**Outcome if missing from request form**
Patient name (Surname & Forename)	Mandatory	Discard
Date of birth	Mandatory	Discard
Hospital number/NHS number	Mandatory	Discard
Date sample taken	Mandatory	Discard

The following information is required on the request forms:

Details (Please note Patient details on sample & request form must match exactly)	**Requirement**	**Outcome if missing from request form**
Patient name (Surname & Forename)	Mandatory	Discard
Date of birth	Mandatory	Discard
Hospital number/NHS number	Mandatory	Discard
Clinical details/ tests required	Mandatory	Discard if we can not ascertain required details
Diagnosis	Desirable	Process
Date and where applicable time (e.g. solid organ rejection cases)	Desirable	Discard if no information on sample tube
Requesting person and place to report.	Desirable	Process and identify requesting clinician/GP, e.g. by contact requesting hospital/GP

In cases of clinical urgency or where it is not possible to obtain repeat samples (i.e. deceased donor samples), the laboratory may agree to process a sample referred without all requested identifiers in accordance with locally documented procedures (copy available on request).

In some cases, prior to processing the sample, the laboratory will need verbal/written clarification that the referring clinician will accept responsibility for processing the requests.

Generated reports will clearly state which identifiers were missing and whether these were from the sample, the request form or both. It is the responsibility of the clinician receiving the report to check the identifiers given to confirm this is the correct patient/donor.

Urgent testing of samples must be by prior arrangement by contacting the laboratory.

Consent

Please note that it is the responsibility of the requester to obtain informed consent for the requested tests. Surplus material s may also be stored for further diagnostic testing to benefit the individual and anonymously for quality control, education and training and approved research and development.

Confidentiality

The laboratories have access to the data and information needed to provide a service that meets the needs and requirements of internal and external customers. The laboratory information system (whether computerised or paper-based) provides for the collection, processing, recording, storage, and retrieval of data, and has documented procedures in place to ensure the confidentiality of patient information and the security of the data during each step of the process. WBS laboratories comply with the data Protection Act 1998 and the Caldicott Principles.

Important Factors That May Affect Histocompatibility & Immunogenetics (H&I) Tests

The following factors may affect any of the H & I tests that are performed by the Laboratory:

Factors that may affect testing	How to minimise effects
Sample storage and transportation temperature	Store and transport samples at ambient temperature.
Sample storage time	Ensure samples arrive within 24 hours of collection.
Anticoagulant	Ensure correct blood tubes are used - see the 'Standard Tests and Samples Required' Table
Immunosuppressant's, e.g. Rituximab, IVIg	Note medication/treatments on sample request form
Low white cell count	Note low count on sample request form

Please contact the Laboratory for help and advice on any of the above.

Standard Tests and Samples Required

Testing Required		Samples Required		
		EDTA	Clotted	Other
Renal/ Pancreas Recipient HLA-typing	New patients & re-type samples	10ml 6ml – hand-written label	20ml	-
Renal Donor Testing	Recipient	40ml 6ml – hand-written label	20ml	-
	Donor – Live	40ml 6ml – hand-written label	-	-
	Donor – Deceased	60ml	-	Lymph/Spleen
Renal Antibody Monitoring Samples	Routine Transplant list Post Transplant Rejection HLA incompatible	-	20ml	-
ABO Titres	Recipient/Donor	6ml	-	-
Corneal graft	-	40ml 6ml – hand-written label	20ml	-
HSCT	Recipient	40ml 6ml – hand-written label	-	-
	Family member	10-20ml	-	-
Disease Association Investigations	HLA-B27 typing	5-10ml	-	-
	Miscellaneous e.g. Coeliac disease, Abacavir sensitivity	10-20ml		-
Platelet investigation	Refractory NAIT (mum) GITP ITP Platelet antibody investigation	40ml	10ml	-
	NAIT (partner)	20ml	-	
	NAIT (foetus/baby)	Contact laboratory for sample requirements		
	Drug – ITP	40ml	20ml	Sample of medication implicated
Transfusion reactions		-	6ml	-
TRALI Investigations		4 x 6ml	4 x 6ml	-

Additional Samples and Testing

Buccal swabs may be accepted by prior arrangement with the Molecular Genetics department, contactable via email: molecular.genetics@wales.nhs.uk or by calling: 01443 622041. On agreement a pack of acceptable swabs and the procedure to follow for sampling will be sent to the requesting Clinician.

WHAIS also offer a range of other investigations for patients by request. For further information please contact Dr T. Rees, Head of WTAIL.

Blood Sample Transportation

Blood samples should normally be transported at ambient temperature and delivered to the Laboratory in as timely a manner as possible and at least within 24 hours of collection. All specimens must be packaged in accordance with the current **Post Office regulations** and European Agreement concerning Carriage of Dangerous Goods by Road Regulations & packing instructions 650 (ADR 2007) to prevent breakage or spillage in transit. They must be, clearly labelled according to the ADR 2007 regulations and display the hazard diamond as illustrated below.

In addition, the statement '**BIOLOGICAL SUBSTANCE CATEGORY B'** must be noted on the packaging. Packaging materials must be suitable for transporting UN3373 Biological substance Category B or UN 2814 as appropriate and addressed to:

Welsh Transplantation and Immunogenetics Laboratory
Welsh Blood Service
Ely Valley Rd,
Pontyclun
CF72 9WB

For other arrangements please contact the Laboratory for help and advice.

Procedures for Specimen Handling

Our procedures for handling blood samples and other specimens are available on request.

Reporting Times

The following target response times relate to working days, where applicable, after the sample/request is received at the laboratory if requested, a copy of the report can be faxed or emailed as soon as it is available.

For urgent requests please contact the laboratory to arrange testing and reporting arrangements.

Service	Report (written unless otherwise stated)	WTAIL Operational lead	Target Turnaround Times (90% within)
Renal Transplantation	Live Donor Work-up Mismatch Grade	Head of Patient Services	5 working days
	Live Donor Work-up typing and crossmatching	Head of Patient Services	10 working days
	Live Donor Final Testing	Head of Patient Services	1.5 working days
	Deceased donor testing	Head of Patient Services	6 hours
Haemopoietic Stem Cell Transplantation	Recipient HLA typing	Head of Molecular Genetics	5 working days
	Potential family donor work-up-matched donors	Head of Molecular Genetics	10 working days from receipt of last sample
	Potential family donor work-up no matched donors	Head of Molecular Genetics	5 working days from receipt of last sample
	Unrelated donor work-up	Head of Molecular Genetics	10 working days from receipt of last sample
Platelet Immunology	Platelet Antibody Investigations – Urgent (verbal report)	Head of Clinical Antibody Testing	1 working day
	Platelet antibody investigations non urgent and written reports for urgent	Head of Clinical Antibody Testing	5 working days
Additional Services	Low resolution HLA type	Head of Molecular Genetics	5 working days
	HLA-B27 typing	Head of Patient Services	10 working days
	HLA antibody investigations - urgent	Head of Clinical Antibody Testing	1 working day

Please note, when successive testing or testing of multiple individuals is required, e.g. typing of potential haemopoietic stem cell transplant donors, the turnaround times may not apply.

Compliments and Concerns

If you have any complaints, or suggestions for improvements, please contact either Dr T. Rees or one of the WTAIL operational leads. Contact can be made via telephone on 01443 622175 and 01443 622178, respectively; by facsimile on 01443 622309; via e-mail using Tracey.Rees2@wales.nhs.uk; or by writing to Dr T. Rees, Welsh Transplantation and Immunogenetics Laboratory, Welsh Blood Service, Ely Valley Road, Talbot Green, Pontyclun. CF72 9WB.

Although we are committed to providing our customers with an excellent service there may be times when we fail to fully meet your requirements. As a learning organisation we firmly believe that every complaint is to be valued as an opportunity to discuss with our customers how the service can be improved.

Terms and Conditions

The WBS standard terms and conditions of service shall apply where a Service Level Agreement has not been signed between WHAIS and the customer. A full set are available on request, the key elements of which are detailed below:

Invoicing

Velindre NHS Trust shall invoice the customer for work undertaken on completion of the individual task, where appropriate. Payment due within 30 days of receipt of invoice.

Any Debts not paid within 30 days shall be considered to be outstanding and may incur interest charges at a daily rate of 0.05%, of the outstanding balance, until the amount has been settled.

Method of payment

The general method of payment shall be in accordance with current policy as stated on the reverse of the Invoice. No receipt will be given unless specifically requested.

Any customer requesting a service supplied by WHAIS shall be deemed to agree to these terms and conditions.

Quality Assurance

WHAIS complies with the WBS Quality Manual, Management Procedures and Standard Operating Procedures (SOPs). A comprehensive Quality Assurance programme monitors and audits all aspects of the service.

Standards of testing are maintained by the rigorous use of internal quality assurance protocols and through participation in appropriate UK National External Quality Assessment Services (UK NEQAS). In addition, WHAIS participates in International Exchange Schemes.

Quality Assessment and External Audit

A copy of last year's participation certificate and results summary is available, on request, for each of the following:

- **UK NEQAS for Histocompatibility and Immunogenetics**
- **University of California, Los Angeles International Cell Exchange**
- **University of California, Los Angeles International HLA DNA Exchange**
- **British Blood Transfusion Society (BBTS) – Platelet and Granulocyte Serology Working Group (Organised by National Institute for Biological Standards and Control (NIBSC))**
- **UK NEQAS for Blood Transfusion Practice**

Accreditation and Regulation

WHAIS laboratories are accredited by Clinical Pathology Accreditation (UK) Ltd. (CPA).

WHAIS is also accredited by the European Federation for Immunogenetics (EFI).

Accreditation categories and techniques covered are available on request.

WHAIS, as part of the WBS, is also regulated by the Medicines and Healthcare Products Regulatory Agency (MHRA).

Further information

More detailed information on any of these services can be obtained, on request.

Please contact Dr T. Rees, Head of WTAIL or one of the following WTAIL operational leads

Miss Emma Burrows, Head of Clinical Antibody Testing
Mrs Sandra Lloyd, Head of Patient Services
Mrs Jennifer Pepperall, Head of Molecular Genetics

Laboratory Contacts for Services Provided

WHAIS Services	Primary Lead	Ext.	Section
Renal and Pancreas Transplantation	Sandra Lloyd	622186	HLA Patient Services
Haemopoietic Stem Cell Transplantation	Jennifer Pepperall	622041	Molecular Genetics
HLA-B27 typing in the spondyloarthropathies	Sandra Lloyd	622186	HLA Patient Services
Miscellaneous Disease Investigations (excluding HLA-B27)	Jennifer Pepperall	622041	Molecular Genetics
HLA Antibody Investigations	Emma Burrows	622031	Clinical Antibody Testing
Platelet Investigations	Emma Burrows	622191	Clinical Antibody Testing
General Service and Research Enquiries	Tracey Rees	622175	Head of WTAIL

Additional Services Hosted by the Laboratory

Service	Primary Lead	Ext.	Website Link
WBMDR	Martin McGregor	622025	WBMDR
UK NEQAS for H&I	Deborah Pritchard	622185	UK NEQAS for H&I

Training

WHAIS has a designated Training Manager supported by a Training Co-ordinator and BSHI Diploma Training Managers. Training incorporates formal, informal, in-house and external courses. Most laboratory staff are graduates and are trained to British Society for Histocompatibility & Immunogenetics (BSHI) Diploma as a minimum. Other qualifications include Ph.D., Dip.RCPath., FRCPath, Royal College of Pathologists Part 1, MSc and FIBMS. Members of WHAIS hold various external training positions (e.g. Supervisor of higher degree students).